

A seed predator drives the evolution of a seed dispersal mutualism

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Although antagonists are hypothesized to impede the evolution of mutualisms, they may simultaneously exert selection favouring the evolution of alternative mutualistic interactions. We found that increases in limber pine (*Pinus flexilis*) seed defences arising from selection exerted by a pre-dispersal seed predator (red squirrel *Tamiasciurus hudsonicus*) reduced the efficacy of limber pine's primary seed disperser (Clark's nutcracker *Nucifraga columbiana*) while enhancing seed dispersal by ground-foraging scatter-hoarding rodents (*Peromyscus*). Thus, there is a shift from relying on primary seed dispersal by birds in areas without red squirrels, to an increasing reliance on secondary seed dispersal by scatter-hoarding rodents in areas with red squirrels. Seed predators can therefore drive the evolution of seed defences, which in turn favour alternative seed dispersal mutualisms that lead to major changes in the mode of seed dispersal. Given that adaptive evolution in response to antagonists frequently impedes one kind of mutualistic interaction, the evolution of alternative mutualistic interactions may be a common by-product.

Keywords: conflicting selection; mutualism; phenotypic selection; secondary seed dispersal; seed predation

1. INTRODUCTION

The outcome of many interspecific interactions is variable. Nowhere is this more striking than when the outcomes of interactions shift from being antagonistic in some populations to increasingly mutualistic in other populations (Thompson & Fernandez 2006; Siepielski & Benkman 2007a; Palmer *et al.* 2008). Understanding why such shifts occur is of particular interest because many mutualisms are thought to have evolved from antagonistic interactions (Thompson 1994). Consequently, identifying the ecological and evolutionary factors responsible for such shifts may provide insight into the evolution and maintenance of mutualisms (Thompson 1994; Bronstein 2001a; Palmer *et al.* 2008).

Seed dispersal mutualisms are of considerable significance because seed dispersal has important fitness consequences, influences spatial genetic variation within and among plant populations, determines rates of recruitment, invasion and range expansion (Nathan & Muller-Landau 2000), and ultimately affects patterns of species coexistence and community structure (Chesson 2000). Not surprisingly, numerous studies have examined how phenotypic selection exerted by seed dispersers influences the evolution of plant reproductive traits (Herrera 2002). However, less attention has been given to the evolutionary effects of seed predators on seed dispersal mutualisms (Herrera 1985; Jordano 1987; Benkman 1995a; Siepielski & Benkman 2007b,c). Most studies on the impact of seed predators on seed dispersal have instead focused on the consumptive effects of seed

predators (e.g. Horvitz & Schemske 1986; García *et al.* 2005). Yet, antagonists are often thought to constrain, impede and possibly lead to the breakdown of mutualisms (Bronstein 2001b; Bronstein *et al.* 2003).

The interactions between limber pine (*Pinus flexilis*) and its primary seed dispersal mutualist, Clark's nutcracker (*Nucifraga columbiana*), and main pre-dispersal seed predator, red squirrels (*Tamiasciurus hudsonicus*), provide an ideal system to investigate the ecological and evolutionary consequences of antagonists on mutualisms. Our previous studies found that red squirrels out-compete Clark's nutcrackers for limber pine seeds (Benkman *et al.* 1984; Siepielski & Benkman 2007b,c). Selection by red squirrels also impedes the evolution of cone and seed traits that facilitate seed harvest and dispersal by nutcrackers, because red squirrels exert selection that conflicts with selection exerted by nutcrackers (Siepielski & Benkman 2007b,c). For example, selection on cone structure by nutcrackers in areas where red squirrels have been absent for 10 000 or more years (the Great Basin) in comparison with areas with red squirrels (the Rocky Mountains) results in a large increase in the seed harvesting rates of nutcrackers with the potential for many more seeds harvested, cached and thus dispersed. Red squirrels therefore act to constrain the mutualism between limber pine and nutcrackers.

The differences in seed availability to nutcrackers between areas with and without red squirrels should also affect seed availability for other species, especially ground-foraging scatter-hoarding rodents. Large-seeded pines such as limber pine (more than 90 mg seeds) are dispersed mostly by corvids (Lanner & Vander Wall 1980; Tomback & Linhart 1990) or scatter-hoarding rodents (Vander Wall 1997, 2003; Hollander & Vander Wall 2004). In fact, scatter-hoarding rodents are apparently the only seed disperser of limber pine in small extremely isolated areas

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2008.0451> or via <http://journals.royalsociety.org>.

where nutcrackers are absent (Tomback *et al.* 2005). Much like nutcrackers, scatter-hoarding rodents benefit pines by moving seeds away from parent plants, which can reduce both density-dependent seed predation and intraspecific competition (Vander Wall 1993). These rodents also cache seeds in the ground where conditions are favourable for germination and seeds are less likely to be found by seed predators (Vander Wall 1993). Thus, cone and seed traits that reduce the foraging rates of red squirrels (cone removal) and nutcrackers (extraction of seeds from cones) should increase seed availability to scatter-hoarding rodents, which may result in rodents becoming more important seed dispersal mutualists. In addition, as the importance of seed dispersal by rodents increases, traits should evolve to facilitate this interaction.

Here, we use observational and experimental approaches to test the hypothesis that geographically variable selection exerted by red squirrels has caused a shift in the relative importance of different limber pine seed dispersers between areas with and without red squirrels. First, we determine whether more seeds fall to the ground in areas with red squirrels than in those without them. Second, we examine how variation in cone and seed traits affects seed availability for ground-foraging rodents by examining how the proportion of seeds that fall to the ground (i.e. which are not harvested by nutcrackers) is related to cone and seed traits under selection by nutcrackers and red squirrels. In making these comparisons between the regions with and without red squirrels, we assume that selection by squirrels and the absence of squirrels accompanied by selection from nutcrackers have driven the observed changes in limber pine cone and seed traits between regions with and without squirrels, respectively. Evidence in support of this assumption include patterns of limber pine cone and seed trait evolution that (i) match those predicted if such selection was important, (ii) is replicated in another species of pine (whitebark pine *Pinus albicaulis*), and (iii) is replicated between areas with squirrels both east and west of the Great Basin (Siepielski & Benkman 2007a). Third, we use a reciprocal transplant seed-tracking experiment to investigate the effect of seed coat thickness on seed fate and dispersal distances in areas with and without red squirrels. Seed coats are over twice as thick in areas with red squirrels as those in areas without them (Benkman 1995a; Siepielski & Benkman 2007b). Although red squirrels exert selection favouring thicker seed coats (Siepielski & Benkman 2007b), an alternative (but not mutually exclusive explanation) is that selection by scatter-hoarding rodents may also favour thicker seed coats, and such selection is stronger where red squirrels are present than absent. This hypothesis would be supported if seeds with thicker seed coats were less likely to be eaten and more likely to be cached (at farther distances) by ground-foraging rodents than seeds with thinner seed coats.

2. MATERIAL AND METHODS

(a) Study system

We studied the interactions between limber pine, Clark's nutcrackers, red squirrels and nocturnal scatter-hoarding rodents in the Great Basin (without red squirrels) and the Rocky Mountains (with red squirrels; figure 1 in the electronic supplementary material) in 2006. All study sites

were located in limber-pine-dominated habitat, with various grasses and sagebrush (*Artemisia*) comprising the understorey. We attempted to control for potential differences in abiotic and biotic conditions using sites located at similar latitudes (39.01–42.52° N; figure 1 in the electronic supplementary material) and elevations (2279–3337 m). Whether pine squirrels (*T. douglasii* and *T. hudsonicus*) were present in the Great Basin in the past 20 000 years is unknown (Grayson 1987; Heaton 1990). However, the large expanses of mostly treeless basins between the mountain ranges in the Great Basin would have prevented pine squirrels from colonizing in the past 10 000 years (Arbogast *et al.* 2001). Given the 'successful' introductions of pine squirrels to other isolated areas (see Benkman *et al.* 2001), we suspect that the absence of pine squirrels from the more forested mountain ranges is the result of biogeographic barriers rather than habitat unsuitability.

Deer mice (*Peromyscus maniculatus*) are ubiquitous in the mountain ranges of western North America, seed dispersers of limber pine (Tomback *et al.* 2005) and other large-seeded pines (e.g. Vander Wall 1997, 2003; Vander Wall *et al.* 2001; Johnson *et al.* 2003; Hollander & Vander Wall 2004), and probably the most important seed dispersing nocturnal rodent in our study areas. We did not follow the fate of cached seeds to determine whether they established. However, studies by Tomback *et al.* (2005) indicated that the caching of limber pine seeds by nocturnal rodents, including *Peromyscus*, contributes to limber pine recruitment. Our results for nocturnal rodents should also be general to diurnal scatter-hoarding rodents (i.e. *Tamias*, *Spermophilus*) common to both study regions (Hall 1981), owing to the generally similar foraging behaviours of different scatter-hoarding rodent species in pine woodlands in western North America (Vander Wall 1997, 2003; Hollander & Vander Wall 2004).

Other seed predators, including insects and several bird species that remove seeds once cones open (e.g. *Poecile gambeli*, *Sitta carolinensis*), are also common to both regions (Hedlin *et al.* 1980; Sibley 2000). Only Steller's jays (*Cyanocitta stelleri*) that are seed predators and dispersers of some pines (Thayer & Vander Wall 2005) are unevenly distributed between areas with and without red squirrels. However, because Steller's jays are common in the Rocky Mountains but mostly absent from the Great Basin (Sibley 2000), Steller's jays should only reduce the likelihood that we would detect less seed fall in the absence of red squirrels. We do not know of any selective agent, including abiotic factors, on cone and seed traits other than pine squirrels and perhaps Steller's jays that differ in a consistent manner between the study sites in the two regions (Siepielski & Benkman *in press*).

(b) Seed fall in areas with and without red squirrels

We used seed traps to compare seed fall between areas with and without red squirrels. We placed two seed traps (aluminium pans (45 × 30 × 8 cm) covered with wire mesh to prevent rodent access) under 15 randomly located trees in forest openings at each of five sites both with and without red squirrels (figure 1 in the electronic supplementary material). We chose trees in forest openings that red squirrels avoid because these are the local habitats where seed removal by nutcrackers (and rodents) are most important; in more densely forested areas, red squirrels remove most of the cones and few seeds are potentially dispersed (Benkman *et al.* 1984). Traps were left from early August, before cones

opened, until the second week of October when few seeds remained in the cones. Seeds in traps were then counted and opened to determine whether they were full (contained female gametophyte) or empty; only full seeds were used in analyses. For each tree, the number of seeds that fell to the ground was estimated as the number of seeds collected in the seed traps multiplied by the inverse of the proportion of the canopy area covered by the traps. We measured the radius of the canopy (r) of each tree to estimate canopy area (πr^2). This assumes that of the seeds that fell, similar proportions fell directly below the canopy in each area. Because limber pine seeds are heavy (more than 90 mg), usually wingless (the only 'wing' present is non-functional) and are unlikely to be dispersed by wind beyond the reach of the canopy (Lanner 1985), this assumption should be justified. The total number of full seeds for a given tree was estimated as the number of cones on the tree multiplied by the mean number of full seeds per cone for that tree. For each tree, we counted (using 10×40 binoculars) the number of cones (cone count repeatability = 0.82; Siepielski & Benkman 2007b) and haphazardly removed two unharvested cones (prior to cone opening) and opened the cones after oven drying them at 60–70°C for more than 2 days to count the number of seeds. We did not attempt to measure within-tree variation (see below) because our analyses rely upon tree means and other studies have found that within-tree variation in cone and seed traits of pines is considerably smaller than among-tree variation (Smith 1968; Elliott 1974), and this was consistent with our observations. The proportion of seeds falling to the ground for a given tree was calculated as the number of seeds falling divided by the number of seeds in the canopy. We used ANOVA to compare the number and proportion of seeds falling to the ground between areas with and without red squirrels.

(c) Seed fall in relation to cone and seed traits and phenotypic selection by nutcrackers

We used regression analyses to examine how the proportion of seeds that fell to the ground from a tree was related to its cone and seed traits. For each of the two cones collected from the 15 trees (as described above), we measured a set of standard traits: cone length; closed cone width; cone mass with seeds removed; peduncle diameter; number of scales along the cone axis; proximal scale thickness; total number of seeds; individual seed mass; and individual seed coat thickness (see Siepielski & Benkman 2007b for measurement details). Because we were interested in how the proportion of seeds falling to the ground was related to overall cone structure, we used principal components analysis based on the correlation matrix of the above cone and seed traits to create a composite variable characterizing cone structure. We focus our analysis on the first principal component (PC1) because our previous studies (Siepielski & Benkman 2007b,c) indicate that most variation (more than 50%) can be accounted for by PC1 and variation in PC1 scores is interpretable in terms of variation in selection exerted by red squirrels and nutcrackers. For this analysis, one site within the region occupied by red squirrels (South Pass, WY; figure 1 in the electronic supplementary material) was treated as a separate category: squirrels absent locally. Red squirrels were absent where traps were placed in all of the other Rocky Mountain sites; however, those trees were isolated individuals, whereas the South Pass site was an isolated stand of several hundred trees. We found no evidence of red squirrels (e.g. cone middens, squirrel

vocalizations) at this site, although red squirrels occupied nearby habitats. We first used ANCOVA to determine whether the slopes between the proportion of seeds falling to the ground and PC1 for the trees at a given site were similar among all sites with red squirrels (the South Pass site was separate) and among all sites without red squirrels.

To further investigate phenotypic selection by nutcrackers on limber pine cone and seed traits, we placed seed traps under an additional 60 trees (a total of 75 trees) at the South Pass, WY study site. We used two measures as a surrogate of tree fitness: the proportion and the number of seeds removed by nutcrackers (Siepielski & Benkman 2007a,b). The proportion of seeds removed by nutcrackers was estimated as one minus the proportion of seeds that fell to the ground (as described above). As in our previous studies (Siepielski & Benkman 2007a,b), we assumed that those seeds not falling to the ground were removed by nutcrackers. Cone and seed traits were standardized to zero mean and unit variance. Individual tree fitness was converted into relative fitness by dividing individual tree fitness by population mean fitness. Selection gradients (β), based on multiple linear regressions, were used to identify the traits under direct selection relative to the effects of other correlated traits (Lande & Arnold 1983). To control for multicollinearity, we examined variance inflation factor (VIF) scores from regression models and correlations between traits; traits contributing to multicollinearity (VIF scores > 10; SAS Institute, Inc. 2003) were removed (the strongest remaining correlation was $r = 0.44$). For this analysis, we also included second-order (γ , quadratic) and cross product (γ_{ij} , correlational) terms to examine nonlinear selection. Univariate selection differentials (i), estimated from regressions between relative fitness and each trait, were used to identify the traits under selection (direct and indirect) by nutcrackers; second-order (c) terms and cubic splines (Schluter 1988) were also examined.

(d) Seed predation and dispersal by nocturnal rodents

To compare the initial fates of seeds removed by nocturnal rodents, we used fluorescent seed tracking (Longland & Clements 1995; Tomback *et al.* 2005) combined with a reciprocal transplant experiment using seeds from areas with (mean seed coat thickness = 0.35 ± 0.0006 mm (s.e.)) and without (mean seed coat thickness = 0.16 ± 0.0005 mm) red squirrels (thick and thin seed coats, respectively) in both areas with and without red squirrels. Seed kernel masses do not differ between areas with (mean = 44.13 ± 0.003 mg (s.e.)) and without (mean = 43.05 ± 0.002 mg) red squirrels, nor is there a correlation between seed coat thickness and seed kernel mass (Siepielski & Benkman 2007b). We also compared seed fates from regions with and without red squirrels to test whether differences in seed coat thickness between the two regions could be related to selection exerted by scatter-hoarding rodents.

Experiments were conducted between September and October 2006 during each of four nights at each of three study sites, both with and without red squirrels (figure 1 in the electronic supplementary material). At a given site and night, we placed 200 seeds coated with fluorescent powder (DayGlo Corp., Cleveland, USA) in an aluminium tray (40×60 cm) under the canopy of each of six randomly selected trees. Each night the seed trays were moved to a different experimental tree at least 500 m from a previously used tree. Experimental trees had no cones, but were

located within local areas with abundant cone crops. This was done to control for potential differences in seed attractiveness associated with differences in natural seed fall. The seeds were placed in Petri dishes glued to sandpaper impregnated with the same coloured fluorescent powder (Tomback *et al.* 2005). Half of the trays had seeds from the areas with red squirrels and the other half had seeds from the areas without red squirrels (different seed sources had different coloured seeds randomly assigned to them); all seeds had been placed in a microwave oven at the highest setting for 20 s to kill the embryos and eliminate the possibility of gene flow. The seeds were placed out by 19.00 hours and then tracked with a UV lamp (Raytector 5-2, 365 nm lamp; Lyman Products, Middletown, USA) between 03.00 hours and just before sunrise. We searched within an approximately 100 m radius of each seed source. We recorded the (i) total number of seeds removed, (ii) number of seeds removed that were immediately consumed (as evidenced by open seeds), and (iii) number of seeds removed that were cached. At each cache, we recorded the substrate, the number of seeds and the distance to the seed source.

To examine differences in the potential importance of scatter-hoarding rodents in areas with and without red squirrels, and in relation to seed coat thickness, we compared the (i) proportion of seeds removed, (ii) proportion of seeds removed, which were immediately consumed, (iii) proportion of seeds removed, which were cached, and (iv) distances to seed caches. We also summarized the results of analyses using the number of seeds removed, consumed and cached when they differ from those using the proportional measures. For each of these response variables, we used general linear models with area (with or without red squirrels) and seed type (thick or thin seed coats) treated as main effects; site was treated as a random effect (nested within area) and interaction terms were included. Models were constructed so that tests of significance for the squirrel presence/absence effect were of a hierarchical structure tested against the mean square of site nested within areas with or without red squirrels. We log transformed some variables to improve normality and variance. Results are presented as mean \pm s.e. throughout.

3. RESULTS

(a) Seed fall in areas with and without red squirrels

There were no differences in mean cone abundance per tree between areas with (117 ± 3 cones) and without red squirrels (108 ± 5 cones; $F_{1,8,9} = 0.15$, $p = 0.71$). However, because there were more seeds per cone in areas without red squirrels than in those with red squirrels, there were significantly more seeds per tree in areas without (6309 ± 365 full seeds) than with red squirrels (4889 ± 184 full seeds; $F_{1,9,2} = 13.90$, $p = 0.005$). Despite this greater seed abundance, nutcrackers removed a significantly greater proportion of the seed crop in areas without red squirrels (0.77 ± 0.23) in comparison to areas with red squirrels (0.51 ± 0.23 ; $F_{1,9,2} = 39.58$, $p = 0.0001$). Consequently, about twice as many seeds fell to the ground (and were available for ground-foraging rodents) in areas with red squirrels in comparison with areas without red squirrels (figure 1a; $F_{1,9,6} = 30.84$, $p = 0.0003$).

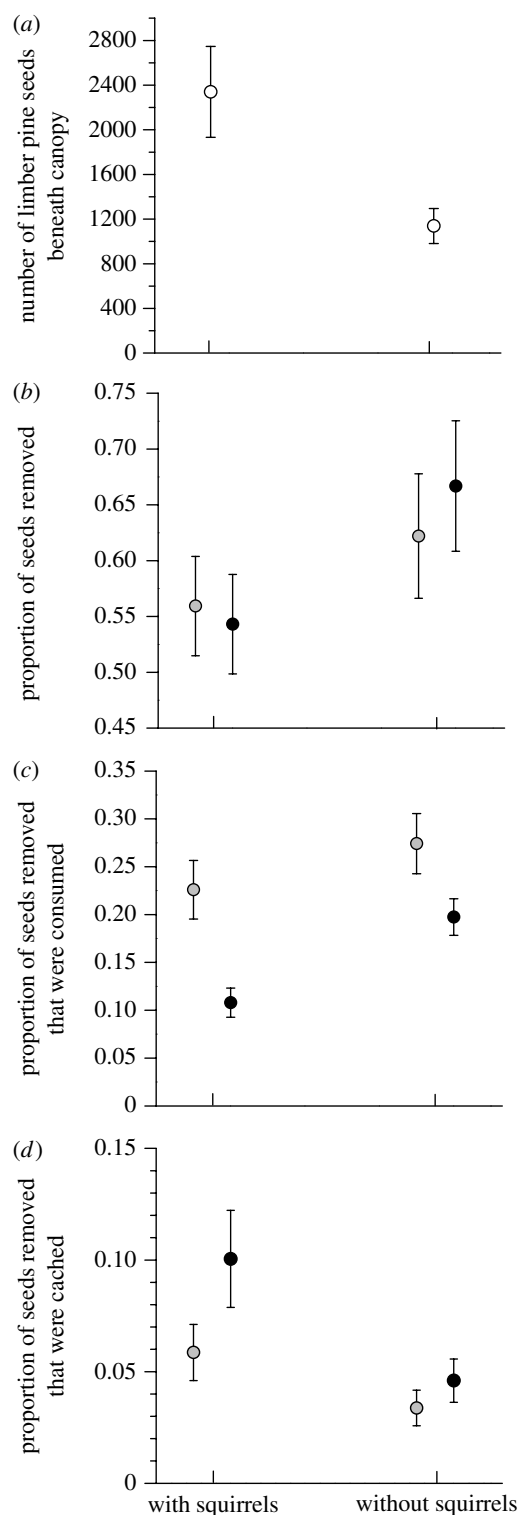


Figure 1. Patterns of limber pine seed fall and removal by nocturnal scatter-hoarding rodents (presumably *Peromyscus*) in areas with and without red squirrels suggest that rodents are more important seed dispersers in areas with red squirrels. (a) Limber pine seed fall, and differences in the (b) proportion of seeds removed, (c) proportion of seeds removed, which were consumed and (d) proportion of seeds removed, which were cached in relation to seed source (grey circles, thin seed coats; black circles, thick seed coats). All results are presented as mean \pm 2 s.e.

(b) Seed fall in relation to cone and seed traits and phenotypic selection by nutcrackers

The PC1 explained much of the variation in cone structure (49%), with increasing values indicating wider

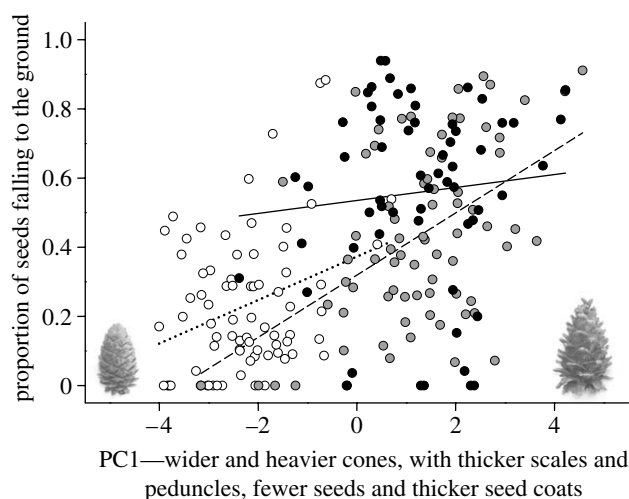


Figure 2. Relationships between the proportion of seeds falling to the ground and the PC1 of nine limber pine cone and seed traits indicate that only in the absence of red squirrels do nutcrackers differentially remove seeds from cones (one minus the proportion of seeds falling) with certain traits. Areas without red squirrels (open circles and dotted line: proportion falling = $37.27 + 6.28$ (PC1), $F_{1,73} = 7.90$, $p = 0.006$), red squirrels absent locally within a region with red squirrels (grey circles and dashed line: proportion falling = $31.94 + 9.01$ (PC1), $F_{1,73} = 22.56$, $p < 0.0001$), and with red squirrels (black circles and solid line (line shown for comparative purposes): proportion falling = $53.53 + 1.88$ (PC1), $F_{1,58} = 0.50$, $p = 0.48$). Shown at the bottom are representative photographs of cones from areas with (right, PC1 score approx. 2.24) and without (left, PC1 score approx. -2.40) red squirrels.

and heavier cones with thicker scales, fewer seeds (and scales) and seeds with thicker seed coats (table 1 in the electronic supplementary material). There were no significant interaction terms for the relationships between the proportion of seeds falling to the ground and PC1 among sites within areas with red squirrels or among sites within areas without red squirrels (ANCOVA interaction terms, $p > 0.05$). Thus, these data were grouped together within each region. In the Great Basin and the South Pass study site (squirrels absent locally), the proportion of seeds falling to the ground increased with increasing values of PC1 (figure 2). Because larger values of PC1 reflect trait combinations indicative of cones that are well defended against predation from red squirrels (Siepielski & Benkman 2007b,c), these results indicate that such seed defences reduce nutcracker seed harvesting efficiency, and thus increase seed fall and availability for scatter-hoarding rodents. By contrast, we found no relationship between the proportion of seeds falling to the ground and PC1 in areas with red squirrels, although the pattern was similar to the other areas (figure 2). However, we had low power to detect a relationship in this regression (based on a retrospective power analysis, power = 0.11), despite sample sizes comparable with the other two regions where power was high (squirrels absent, power = 0.79; squirrels absent locally, power = 0.99). This latter comparison nevertheless suggests that factors other than variation in cone structure contribute to the observed patterns of seed fall.

Multiple regression analyses show that the target of selection by nutcrackers at South Pass, WY, was the

number of seeds per cone, with nutcrackers preferentially removing a greater proportion or number of seeds from cones with more seeds (table 1); results were similar for both measures of tree fitness. Selection (direct and indirect combined) by nutcrackers favoured trees having cones with more seeds and scales, thinner scales and seeds with thinner seed coats (table 1). We found no evidence of nonlinear selection (not shown).

(c) Seed predation and dispersal by nocturnal rodents

A greater proportion of experimental seeds were removed in areas without red squirrels than in those with red squirrels (figure 1b; $F_{1,4.3} = 14.90$, $p = 0.02$); the proportion of seeds removed was not related to seed coat thickness (figure 1b; $F_{1,182} = 0.25$, $p = 0.61$). Of the seeds removed, a greater proportion was consumed and a smaller proportion was cached in areas without red squirrels than in those with red squirrels (figure 1c: $F_{1,4.2} = 22.56$, $p < 0.008$; figure 1d: $F_{1,4.0} = 13.46$, $p < 0.02$, respectively). *Peromyscus* also consumed a greater proportion of seeds with thin seed coats than thick ones (figure 1c; $F_{1,171} = 30.93$, $p < 0.001$), and cached a greater proportion of seeds with thick seed coats than thin ones (figure 1d; $F_{1,167} = 13.51$, $p = 0.0003$). No interaction term was significant for any of the above models (all $p > 0.05$), which indicates that the effects of seed coat thickness did not depend on whether red squirrels were present or absent. However, if we used the number of seeds consumed, we did detect a significant interaction between seed coat thickness and red squirrel presence or absence ($F_{1,171} = 6.61$, $p = 0.01$). Presumably this reflects the observation that seeds with thick seed coats were less likely to be consumed in areas with red squirrels ($F_{1,171} = 20.49$, $p < 0.001$), but not in areas without them ($F_{1,171} = 1.77$, $p = 0.19$). There were no differences in the proportion of seeds removed that were subsequently accounted for between areas with and without red squirrels ($F_{1,4.1} = 3.17$, $p = 0.15$), which indicates that our ability to relocate removed seeds did not differ among regions with and without red squirrels. Overall, the ratio of the proportion of seeds cached relative to the proportion of seeds consumed ('benefits'/costs') was almost four times greater in areas with red squirrels (0.74 ± 0.06) than in those without red squirrels (0.20 ± 0.06 ; $F_{1,4.6} = 21.14$, $p < 0.007$). These ratios were the greatest for seeds with thick seed coats in areas with red squirrels (ratio: 1.11 ± 0.08) and the lowest for seeds with thin seed coats in areas without red squirrels (0.16 ± 0.08), both of which are the nominal seed coat thicknesses for their respective areas.

Rodents cached seeds slightly farther in areas with red squirrels relative to areas without red squirrels (figure 3; 10.55 ± 0.76 m and 9.85 ± 0.60 m, respectively; $F_{1,5.4} = 19.82$, $p = 0.005$). Seeds with thick seed coats were dispersed approximately three times farther than seeds with thin seed coats (figure 3; 14.35 ± 0.57 m and 5.74 ± 0.36 m, respectively; $F_{1,167} = 180.74$, $p < 0.001$). The significant interaction term ($F_{1,167} = 18.80$, $p < 0.0001$) presumably occurred because seeds with thick seed coats were dispersed approximately four times farther (16.41 m) than seeds with thin seed coats (4.68 m) in areas with red squirrels (figure 3a; $F_{1,167} = 145.99$, $p < 0.001$), whereas seeds with thick seed coats were only dispersed approximately twice as far (12.70 m) as seeds

Table 1. Pairwise and multiple linear regression analyses of phenotypic selection exerted by Clark's nutcrackers owing to seed harvesting on limber pine in South Pass, WY ($n=75$ trees). (Two components of tree fitness were evaluated: the proportion of seeds removed and the total number of seeds removed. Tests of significance were based on 1000 bootstrap replicates. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.0001$.)

trait	selection based on proportion of seeds removed		selection based on total number of seeds removed	
	pairwise linear regression t (\pm s.e.)	multiple regression β (\pm s.e.)	pairwise linear regression t (\pm s.e.)	multiple regression β (\pm s.e.)
cone length	0.03 (0.06)	0.04 (0.06)	0.17 (0.09)	0.02 (0.08)
cone width	-0.08 (0.06)	-0.09 (0.06)	-0.02 (0.10)	-0.003 (0.08)
cone mass	-0.06 (0.06)		-0.03 (0.10)	
number of scales	0.16 (0.05)**		0.23 (0.08)**	
proximal scale thickness	-0.20 (0.05)***	-0.07 (0.07)	-0.22 (0.08)**	-0.07 (0.11)
peduncle diameter	-0.13 (0.05)**		-0.16 (0.08)	
total number of seeds	0.18 (0.04)***	0.12 (0.05)*	0.48 (0.07)***	0.45 (0.08)***
seed mass	-0.14 (0.05)*	-0.04 (0.06)	-0.21 (0.07)**	-0.06 (0.06)
seed coat thickness	-0.18 (0.04)***	-0.09 (0.07)	-0.17 (0.07)*	-0.02 (0.090)

with thin seed coats (6.68 m) in areas without squirrels (figure 3b; $F_{1,167}=45.22$, $p < 0.0001$).

Most caches were made in the open and not under adult limber pines, with over 80% of the cached seeds buried in soil and under plants (mostly grasses and sagebrush; figure 2 in the electronic supplementary material). The proportion of different cache substrates used did not differ between areas with and without red squirrels for the different seed types ($\chi^2_9=13.29$, $p=0.15$). The few recognizable tracks left were 17–20 mm long and consistent with the hind-foot lengths of *Peromyscus* (Tomback *et al.* 2005). The mean number of seeds per cache (2.7 ± 0.1) was also consistent with the size of *Peromyscus* caches of similarly sized pine seeds (Vander Wall 1992; Vander Wall *et al.* 2001). Thus, we assumed that most seed removal was by *Peromyscus*.

4. DISCUSSION

Our results indicate that adaptive evolution of limber pine cone structure in response to selection by red squirrels has contributed to a shift in the importance of two seed dispersers between regions with and without red squirrels. Where red squirrels are absent, nutcrackers are the principal seed dispersers while scatter-hoarding rodents probably provide minimal seed dispersal. Here, relatively few seeds fell to the ground because nutcrackers harvested most of the seeds, and the proportion of seeds consumed by rodents greatly exceeded the proportion of seeds cached. By contrast, where red squirrels are present, proportionately more seeds fell to the ground because nutcrackers are less efficient on the well-defended cones, and rodents provide a greater service as seed dispersers (e.g. here the ratio of seeds cached to consumed is nearly seven times greater than that in areas without squirrels). This geographical shift in the importance of different seed dispersers suggests that seed predators can have an important role in driving the evolution of seed dispersal mutualisms.

This happens in at least two ways. First, increased seed defences in response to selection from seed predation by red squirrels reduce the proportion (and number) of seeds harvested by nutcrackers, which increases seed fall and thus seed availability for scatter-hoarding rodents. For example, traits such as large, thick cone scales and fewer

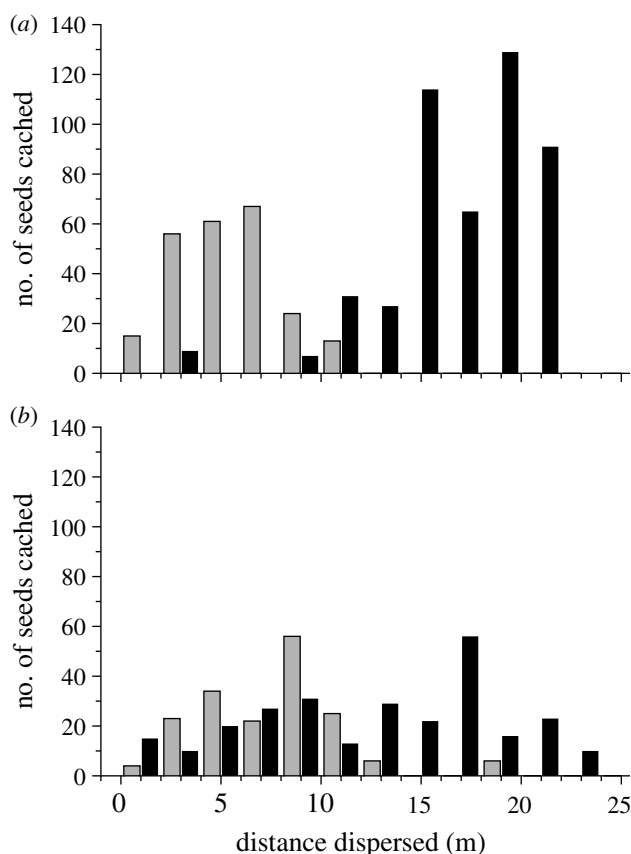


Figure 3. *Peromyscus* cache more seeds with thicker seed coats farther distances. These differences are more pronounced (a) in areas with red squirrels than (b) in areas without them (black bars, thick seed coats; grey bars, thin seed coats).

seeds (e.g. larger values of PC1; figure 2), which deter seed predation by red squirrels also reduces the rate at which nutcrackers remove seeds (Siepielski & Benkman 2007b,c). A result is that nutcrackers harvested approximately 80% of limber pine seeds in areas without red squirrels but only harvested approximately 50% of the seeds from trees in areas with red squirrels (and where red squirrels did not harvest cones). Consequently, about twice as many seeds fell to the ground and became available for seed dispersal by scatter-hoarding rodents in areas with red squirrels than in areas without red squirrels

(figure 1a). This increased seed abundance presumably explains why scatter-hoarding rodents cache a greater proportion and immediately consume a lower proportion of seeds in areas with red squirrels relative to areas without red squirrels (figure 1c,d, respectively). Thus, what appears to be critical in shifting the interaction between limber pine and scatter-hoarding rodents from antagonistic to increasingly mutualistic is the number of seeds that fall to the ground or seed abundance. Such conditional mutualisms are often a consequence of variation in the abundance of partners (Bronstein 1994), and other studies examining how seed abundance affects the outcome of seed dispersal interactions have shown similar patterns (Theimer 2005). For example, nutcrackers (Siepielski & Benkman 2007a) and other seed-dispersing rodents (Vander Wall 2002; Jansen *et al.* 2004) apparently shift from being antagonistic during small seed crops, because they cache few seeds, to being increasingly mutualistic when large seed crops are produced and many seeds are cached. While in these studies the variation in resource abundance was temporal (e.g. masting), our spatial comparison reveals similar patterns among areas producing large seed crops.

The second way that selection by red squirrels affects the potential seed dispersal mutualism between scatter-hoarding rodents and limber pine is via the red squirrel's evolutionary effect on seed coat thickness. Selection exerted by red squirrels favouring thicker seed coats (Siepielski & Benkman 2007b) promotes greater seed dispersal by scatter-hoarding rodents because seeds with thicker seed coats are more likely to be cached (figure 1c) and dispersed farther by scatter-hoarding rodents (especially in areas with red squirrels) than seeds with thinner seed coats (figure 3). Scatter-hoarding animals typically disperse larger seeds with thicker seed coats farther distances both among (Forget *et al.* 1998; Zhang *et al.* 2004; but see Vander Wall 2003) and within species (Jansen *et al.* 2004; but see Brewer & Webb 2001). Other species of large-seeded pines, which apparently rely on scatter-hoarding rodents for seed dispersal (e.g. Johnson *et al.* 2003; Vander Wall 2003), also co-occur with other tree squirrels (Benkman 1995b) and have very thick seed coats (e.g. Johnson *et al.* 2003). This suggests that selection by scatter-hoarding rodents and tree squirrels have contributed to the evolution of their exceptionally thick seed coats. Conversely, the evolution of thinner seed coats in areas without red squirrels (Benkman 1995a; Siepielski & Benkman 2007b) indicates that rodents have probably had a relatively minor impact on limber pine recruitment in areas without red squirrels.

5. CONCLUSIONS

Antagonists are often thought to break down or constrain mutualisms (Bronstein 2001b; Bronstein *et al.* 2003). Consistent with this prediction, our observational results indicate that selection exerted by red squirrels for increased seed defences impedes the seed dispersal mutualism between limber pine and nutcrackers. However, our experimental results also indicate that the potential for seed-caching rodents to act as mutualists increases while the strength of the mutualism between pines and nutcrackers decreases. Although rodents disperse seeds, they do so but a fraction of the distance

nutcrackers disperse seeds (a few tens of meters (figure 3; Johnson *et al.* 2003; Vander Wall 2003) versus a few hundreds of meters to upwards of 22 km (Vander Wall & Balda 1977; Tomback 1998), respectively), which may have consequences for seed-mediated gene flow (Jordano *et al.* 2007). These differences in seed dispersers between areas with and without red squirrels also represent a shift from relying on primary seed dispersal in areas without red squirrels, to relying more on secondary seed dispersal in areas with red squirrels.

In summary, our results reveal that one previously unrecognized pathway for the evolution of a mutualism is the weakening of another mutualism by an antagonist. Although some mutualisms such as ant protection are conditioned on antagonists (i.e. herbivores) being present (Palmer *et al.* 2008), we are not aware of the examples where evolutionary changes imposed by an antagonist (e.g. red squirrels) cause what is otherwise usually another antagonist (e.g. scatter-hoarding rodents) to become increasingly more mutualistic as the importance of the primary mutualist (e.g. nutcrackers) weakens. Given that mutualisms are widespread in ecological communities replete with antagonists (Bronstein 2001b), our results suggest that much insight can be gained by considering alternative outcomes of the constraining effects of antagonists on mutualisms.

All research conformed to the guidelines set forth by the University of Wyoming Animal Care and use committee.

We thank N. Bennett, A. Brody, K. C. Burns, R. Irwin, T. Parchman, J. N. Thompson, L. Santisteban, S. Vander Wall and an anonymous reviewer for their helpful comments on earlier drafts, and the NPS and USFS for their help. Without the support of T. Siepielski, this research would not have been possible. Our research was supported by the NSF (grant DEB-0515735).

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